The Friends of Nachusa Grasslands
2023 Scientific Research Project Grant Report
Due June 30, 2024

Please answer the following questions with clearly written summaries to give Nachusa Friends' science committee members, officers, and board members a good idea of what you accomplished using your grant money. Unless you object to the Friends doing so, your report will be uploaded into the science section of the Friends' website: nachusagrasslands.org.

1. Please save this form to your desktop with a unique file name that includes "Friends 2022 Science Grant Report" and your last name.
2. Complete the form using the headings in bold as your guide.
3. Save the file as a Word document or a PDF.
4. Attach the file to an e-mail, and send it to: nachusafriendsscience@gmail.com no later than June 30, 2024
5. The subject of the e-mail should be "2023 Scientific Research Grant Report" and your last name.
6. If you have not completed your work, please submit this form anyway by the June 30 deadline and plan to contact Friends after your project is complete so that we may learn from and publicize the outcomes as appropriate.

Name: Pallavi Singh
Address: 359 Montgomery Hall, Northern Illinois University, DeKalb, IL-60115
Phone: 815-753-7839 **Current E-mail:** psingh1@niu.edu

2023 grant amount: $1,850

Please answer the following questions with 1- to 2- sentence summaries:

Research Project Topic: Identifying Factors that Influence Bison Health

Research Project Purpose: The goal of this study is to assess the intestinal microbial population of the bison and the various factors that influence it. These factors include seasonal, temperature, and dietary variations which have direct implications on the animal's health. These impact the growth development of the animal and identifying factors that influence bison health will allow us to propose practices that improve their health for continued prairie restoration efforts.

Research Project Outcomes to date: We have collected fecal and water samples year-round from various sites in Nachusa and during the annual round-up, as a result, banked close to 1000 samples thus far. For assessing, the intestinal microbial ecology and pathogen status we have isolated community DNA from all the fecal samples above. We

assessed bacterial pathogen status from a set of samples collected during the winter and summer seasons. We have performed long-term analysis of free-ranging Bison at Nachusa Grasslands based on 16S rRNA genes and found that overall, the microbial composition at the phyla remains similar (**Fig. 1a**). However, it is important to note that while the phyla-level composition remains similar, there may still be changes or fluctuations at lower taxonomic levels (e.g., species or genera) that are not evident when looking solely at the phyla. These fine-scale changes can have functional implications for the bison and their interactions with their environment. Therefore, on further analysis of community level diversity the composition differed significantly (PERMANOVA; $p<0.001$) between the years (genus, **Fig. 1b**). Interestingly, we found that the communities of 2018 were most distinct from remaining years whereas 2020 was closest to 2021 based on Similarity percentage (SIMPER) analysis. This change predominantly was due to *Ruminococcaceae* and *Bacteriodales*, both of which have been linked to improved gut health. Improved gut health will lead to improved bison health and changes in grassland species with feedback from bison, soil, and plant microbial interaction. Additionally, our analysis from four seasons including Fall, Spring, Summer and Winter indicate that the seasonal changes affect the bison gut microbiome (**Fig. 1c**). The fecal communities differed significantly (PERMANOVA; $p<0.001$) across seasons where Summer and Spring samplings were found to be more diverse and richer in bacterial species (**Fig. 1c**) as compared to Fall and Winter. At the genus level we found that rc4-4 and *Lysinibacillus* to dominate in Spring and Summer season and are linked to positive health association. The change in diet may contribute to gut microbiome differences observed in our studies. Bison forage on C4 grasses during late spring and summer whereas C3 during cooler temperature leading to increased protein availability to them These seasonal changes support our hypotheses and work that changes in access to prairie composition affects the animal microbiome and consequently animal health.

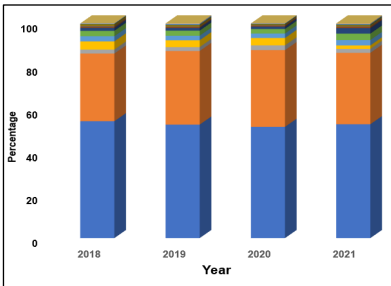

Fig. 1a. Phyla analysis of Bison fecal microbial communities from 2018-2021

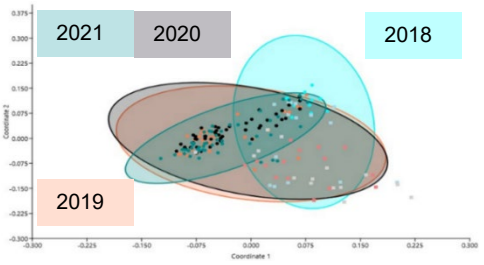

Fig. 1b. Fecal microbial communities clustered significantly ($p<0.001$) clustered separately from each year

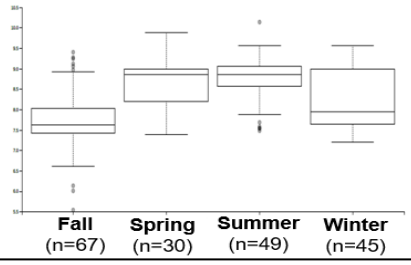

Fig. 1c. Higher diversity and richness were observed in fecal microbial communities from Winter, and Summer as compared to Fall and Spring, samplings.

Describe how the grant funds you have received from the Friends of Nachusa Grasslands have been used in regard to the above topic, purpose, and/or outcomes: Funds from the 2023 grants allowed us to support travel of several students for sample collection as well as provide research and training opportunity to these students, listed table below. These students gained experience in field, and lab (microbial and molecular) techniques.

Students	
Undergraduates	***Graduates/Personnel***
Samantha Mallinder	Pallavi Singh
Yousuf Khan	Laurie Spencer
Mark Figueroa	Ritesh Ray
Aleena Kallan	Jeanine Sitz

Describe how your project has benefited the work and goals of Nachusa Grasslands: The results of the study will reflect upon Bison herd management as our goal is to holistically study animal, and water microbiome to decipher microbial community interplay in the ecosystem. Our year-round fecal and water sample collection has reflected on microbial community variation in Bison host by 16S sequencing. Augmenting Bison growth performance and health is the long-term goal of the project.

Describe how your findings can be applied to challenges in management practices for restoration effectiveness and species of concern: Various factors affect health and growth of Bison in the wild. A well-established and important factor my group is focused on is the intestinal microbial community of the Bison. These communities have important functions of food digestion and protecting the host from various pathogens. Therefore, are important in supporting the health of the animal, however, they can be easily disturbed by a multitude of factors. The goal of our research is to study these factors and provide information on the significantly influential factors identified for instance water or diet. These will enhance our understanding of Bison herd management, which in turn will support prairie restoration efforts.

Please list presentations/posters you have given on your research:

1. L. Spencer, D.A. Nuccio, and P. Singh. 2023. Molecular Characterization of Intestinal Microbiome in Bison at Nachusa Grasslands. Nachusa Science Symposium. Franklin Grove, IL. Oral Presentation.
2. S. Mallinder, R. Ray, and P. Singh. 2023. Determining the Presence of *Salmonella* in Bison in Commercial and Conservational Settings. CURE, NIU. Poster.
3. Y. Khan, R. Ray, and P. Singh. 2023.Molecular detection of bacterial pathogen, *Clostridium perfringens,* in Bison. CURE, NIU. Poster.
4. Y. Khan, R. Ray, and P. Singh. 2023. Molecular detection of bacterial pathogen, *Clostridium perfringens,* in Bison. CURE, NIU. Oral Presentation.
5. L. Spencer, D.A. Nuccio, and P. Singh. 2023. Molecular Characterization of Intestinal Microbiome in Bison at Nachusa Grasslands. Phi Sigma Symposium, NIU. Oral Presentation.
6. H. Tyszka, L. Spencer, E. R. Gaillard and P. Singh. 2023. Identification of Intestinal Parasites in North American Wild Bison. Phi Sigma Symposium, NIU. Oral Presentation

Have you submitted manuscripts to scientific journals? If so, which ones? If not, do you anticipate doing so? (Please send digital copies of published articles to the Friends so that we can learn from your work.**)**

We haven't submitted any research articles, yet. Yes, we anticipate submitting journal articles within the next year.

What follow-up research work related to this project do you anticipate (if any)?
<u>Optional</u>: Suggestions for improving the application and award process for future Friends of Nachusa Grasslands Scientific Research Grants: